



NCI Center
for
Cancer Research
*Reducing the Burden of Cancer Through
Exploration, Discovery and Translation*



Design, Development and Delivery of Recombinant Vaccines for the Therapy of Human Carcinomas

Jeffrey Schlom, Ph.D.
Laboratory of Tumor Immunology and Biology
Center for Cancer Research
National Cancer Institute, NIH



Recombinant Vaccine Programmatic Effort

DISCOVERY:

Laboratory of Tumor Immunology
and Biology, Center for Cancer
Research, NCI

J. Hodge	J. Greiner
H. Sabzevari	A. Tsang
D. Grosenbach	S. Kashmiri
S. Abrams	P. Arlen
J. Schlom	J. Gulley

DEVELOPMENT:

Collaborative Research and
Development Agreement
(CRADA): Therion/NCI

Cancer Therapy Evaluation
Program (CTEP), NCI

DELIVERY:

COLORECTAL/PANCREATIC/LUNG CLINICAL TRIALS

- Georgetown (J. Marshall)
- Fox Chase (M. von Mehren)
- Duke (K. Lyerly)

PROSTATE CANCER CLINICAL TRIALS

- Dana Farber (D. Kufe/P. Eder)
- Center for Cancer Research, NCI
(P. Arlen, J. Gulley, W. Dahut, N. Coleman,
K. Camphausen)
- ECOG (H. Kaufman, R. DiPaola, L. Weiner)

BREAST CANCER CLINICAL TRIALS

- Dana Farber (D. Kufe)
- Center for Cancer Research, NCI
(C. Kasten-Sportes, R. Gress,
P. Arlen)

INTRATUMORAL TRIALS

Melanoma

- Columbia (H. Kaufman)

Hypotheses

- **Tumor Associated Antigens (TAA) are, by definition, either weakly immunogenic or functionally non-immunogenic.**

Cancer Vaccine Targets

Proteins/peptides that are:

- overexpressed in tumors vs. normal tissues

CEA, MUC-1

- overexpressed in tumors and non-vital organs

PSA

Carcinoembryonic Antigen (CEA)

- ◆ 180,000d glycoprotein
- ◆ homoadhesion molecule
- ◆ implicated in the metastatic process
- ◆ Distribution
 - **Carcinoma:**
 - 95% of colorectal , gastric, pancreatic
 - 50% of mammary
 - 70% of non-small cell lung
 - Others: e.g., squamous cell carcinoma of head & neck, cervical carcinoma
 - **Normal:**
 - Extensive in fetal gut
 - Low levels in normal colonic mucosa

Hypotheses

- **Tumor Associated Antigens (TAA) are, by definition, either weakly immunogenic or functionally non-immunogenic.**
- **Vaccine strategies must be developed in which the presentation of these TAAs to the immune system results in far greater activation of T cells than is being achieved naturally in the host.**

Strategies

- 1. Mode of Delivery of the Vaccine**
 - place the gene for the tumor antigen into a vector
- 2. Diversified Vaccine Prime and Boost**
- 3. T-cell Costimulation**
 - these molecules are essential for vigorous T-cell activation
 - place costimulatory molecule into vaccine vector
- 4. Alter the a.a. sequence of the tumor antigen to enhance the immune response “epitope enhancement”**
- 5. Cytokines — biologic adjuvants**

Recombinant Vaccine Vectors

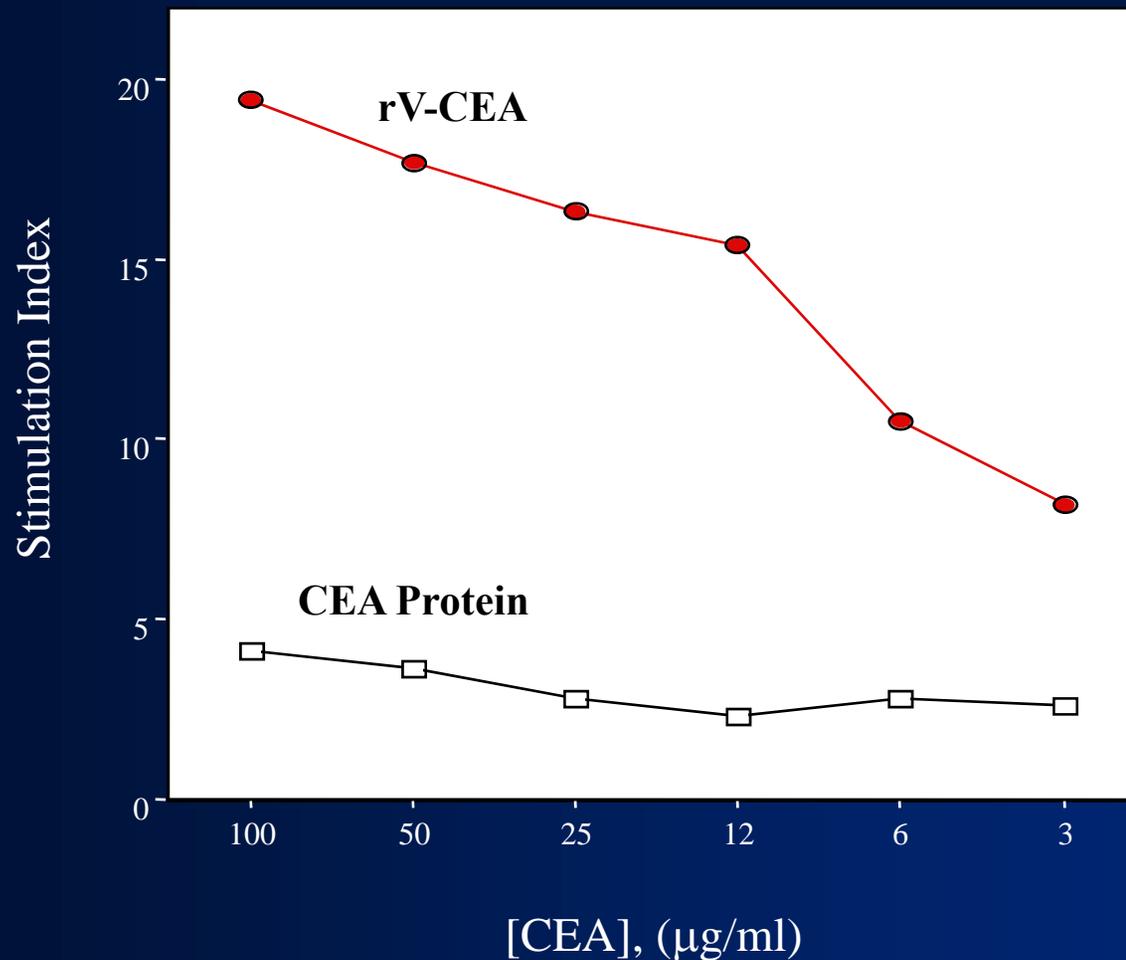
- Pox vectors

- **Vaccinia (rV-)** elicits a strong immune response

- host induced immunity limits its continuous use

- MVA (replication defective)

Comparison of T-Cell Responses in CEA Transgenic Mice Vaccinated with CEA Protein vs. rV-CEA



Recombinant Vaccine Vectors

- Pox vectors

- **Vaccinia (rV-)** elicits a strong immune response

- host induced immunity limits its continuous use

- MVA (replication defective)

- **Avipox (fowlpox rF, ALVAC)**

- derived from avian species

- safe; does not replicate

- can be used repeatedly with little if any host neutralizing immunity

- **Can insert multiple transgenes**

- **Do not integrate into host DNA**

- **Efficiently infect antigen presenting cells including dendritic cells**

Phase I Studies: CEA Vaccines

- **rV-CEA**
 - Safe
 - Induction of T-cell responses specific for CEA
- **Avipox-CEA**
 - Safe
 - Induction of T-cell responses specific for CEA
 - Demonstration that CEA-specific T cells can kill tumor cells expressing CEA

Preclinical Studies: Diversified Prime and Boost Strategy

- rV-TAA (V) : prime vaccination
- Avipox-TAA (A) : booster vaccination

VAA > VVV

VAA > AAA

TAA = tumor-associated antigen

**Diversified prime and boost is more efficacious than
the continued use of one vaccine**

Phase II Study: Vaccination of Patients with Metastatic* CEA-Expressing Carcinomas

- To define if a Diversified Vaccine Prime and Boost Strategy leads to increases in CEA-specific T-cell responses
- Vaccines: rV-CEA (V) and Avipox-CEA (A)

Randomized (n = 9/cohort):

Cohort 1: V-A-A-A

Cohort 2: A-A-A-V

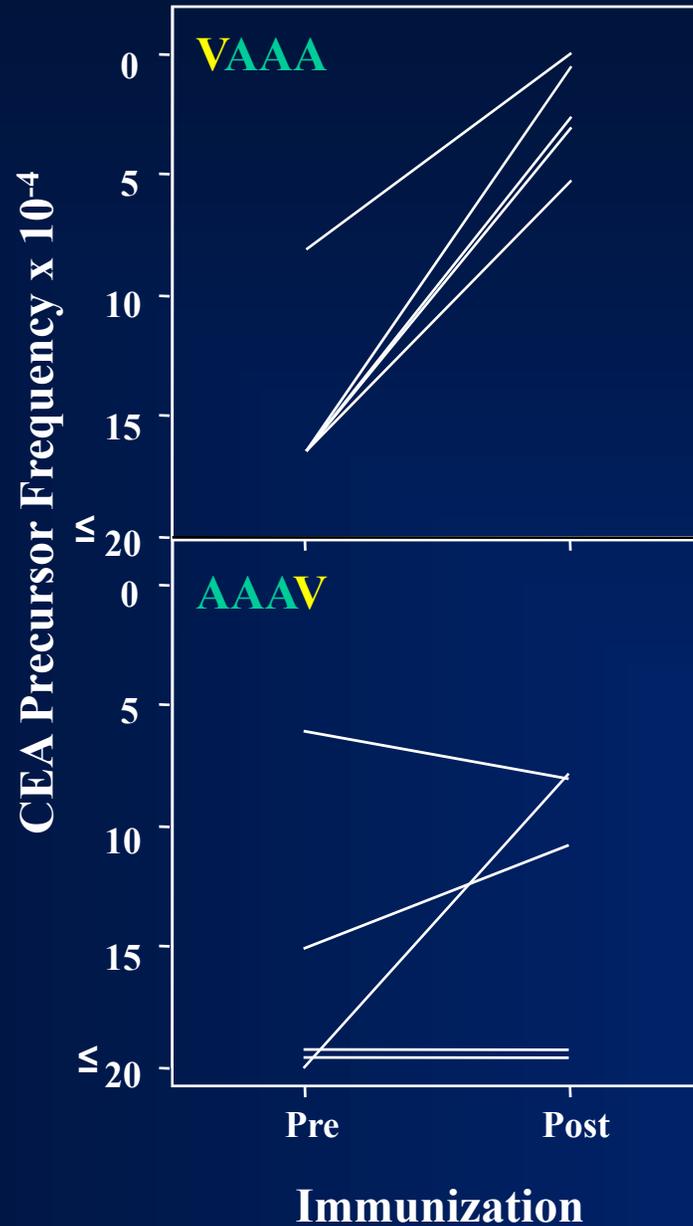
- Define CEA responses to 9 mer CEA peptide using an overnight ELISPOT assay

*Patients received from 2 to 6 prior therapies.

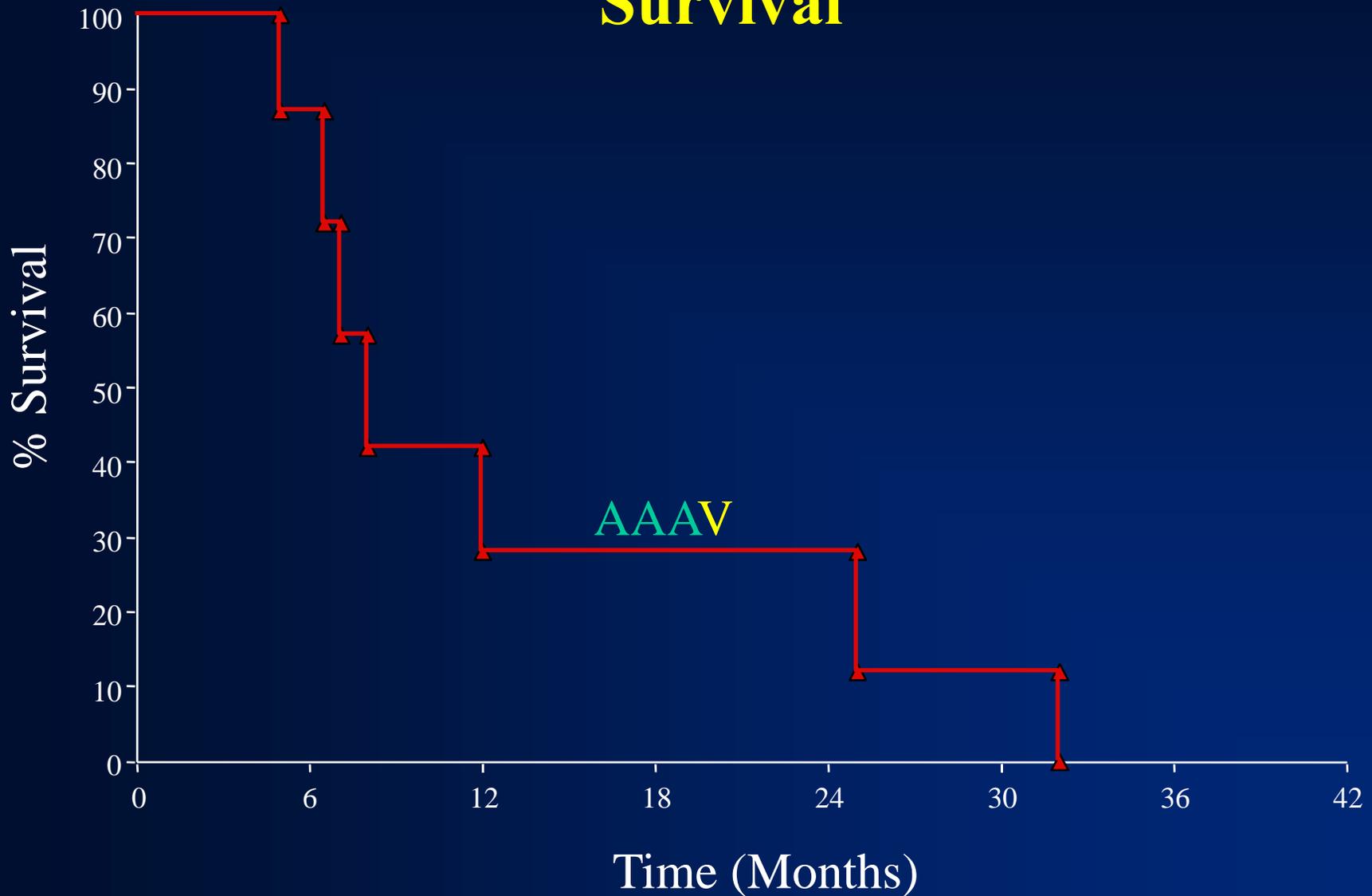
CEA and Flu Precursor Frequencies Following Vaccinations with rV-CEA(V) and Avipox-CEA(A) ± GM-CSF

<u>Patient</u>	<u>Vaccination</u>	<u>Precursor Frequency</u>	
		<u>Flu</u>	<u>CEA</u>
3	Pre	1/75,000	<1/200,000
	V	1/11,000	1/116,000
	V-A	1/62,000	1/71,000
	V-A-A	1/75,000	1/66,000
	V-A-A-A	1/85,000	1/61,000
	V-A-A-A-A (+GM-CSF)	<u>1/86,000</u>	<u>1/19,000</u>
		$\Delta - 0.8x$	$\Delta \geq 10.5x$

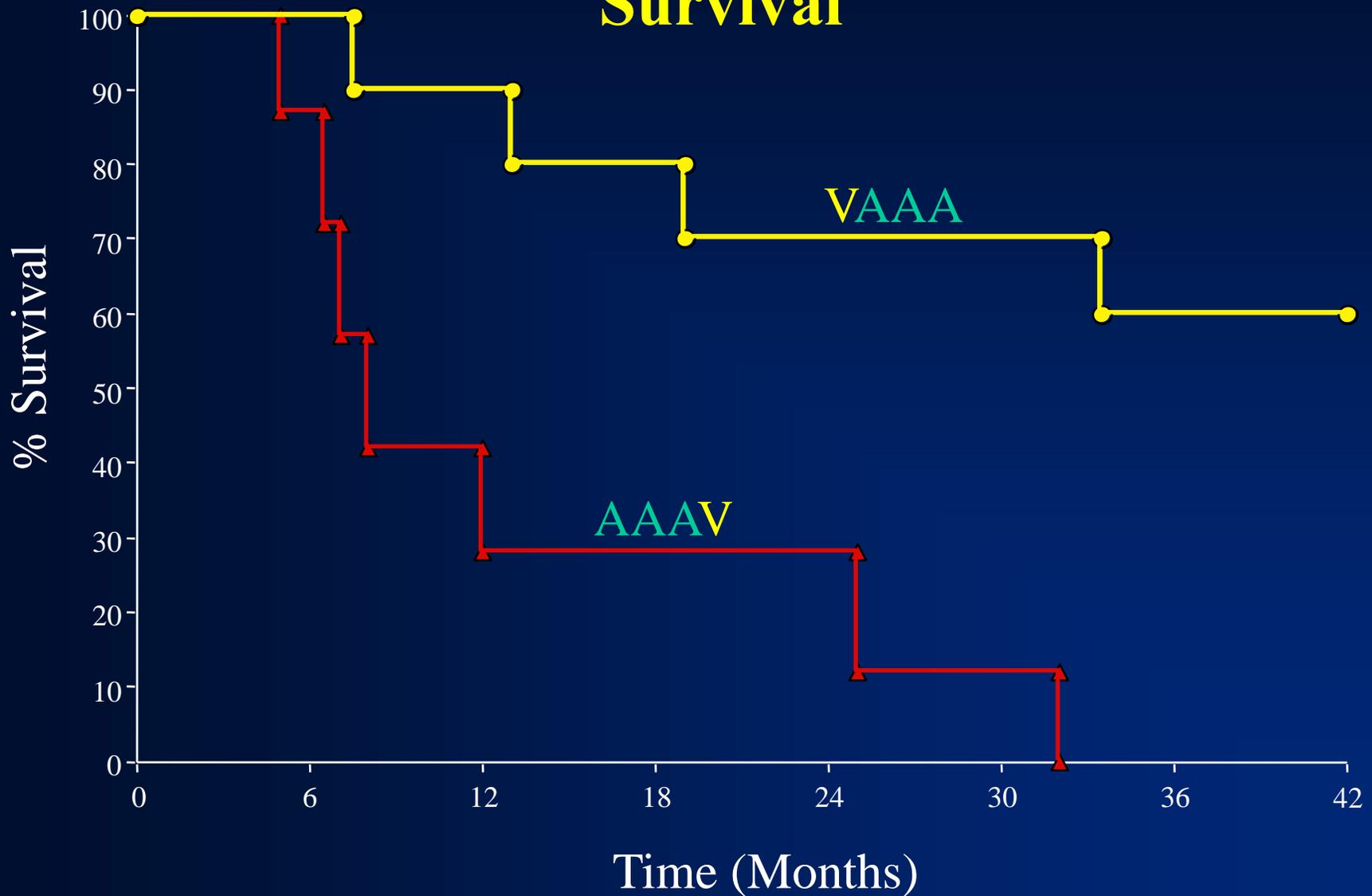
Advantage of Primary Vaccination with rV-CEA (V) and Boosting with Avipox-CEA (A)



Randomized Phase I/II Arms of the Diversified Vaccination of rV-CEA (V) and Avipox-CEA (A): Survival



Randomized Phase I/II Arms of the Diversified Vaccination of rV-CEA (V) and Avipox-CEA (A): Survival



Statistical Analyses: Randomized Cohorts

V=rV-CEA A=avipox-CEA

- Patients in the VAAA cohort had a statistically significant (**p<0.01**) increase in CEA specific T cells (post vs. pre-vaccination) as compared to patients in the AAAV cohort.
- Treatment with VAAA resulted in longer survival than treatment with AAAV (**p=0.05**).
- Survival duration was unrelated to pre-vaccination CEA specific T cell levels (**p=0.77**).
- **The generation of CEA specific T cell responses was associated with increased survival (p=0.03) after accounting for disease status.**

Antigen Cascade

VAAA vs. AAVV CEA Vaccine Trial

<u>Patient 15</u>	<u>Flu</u>	<u>CEA</u>	<u>MUC-1</u>	<u>Her2/neu</u>	<u>p53 103</u>	<u>p53 139</u>	<u>EP-CAM</u>
pre vac	1/23,316	<1/200,000	<1/200,000	<1/200,000	<1/200,000	<1/200,000	<1/200,000
post 1	1/37,500	1/75,000	1/85,714	1/66,667	1/46,154	1/120,000	1/46,154
post 2	1/28,571	1/35,294	1/50,000	1/66,667	1/37,500	<1/200,000	1/75,000

Patient 15 received rV-CEA primary vaccination and avipox-CEA booster vaccination.

Strategies

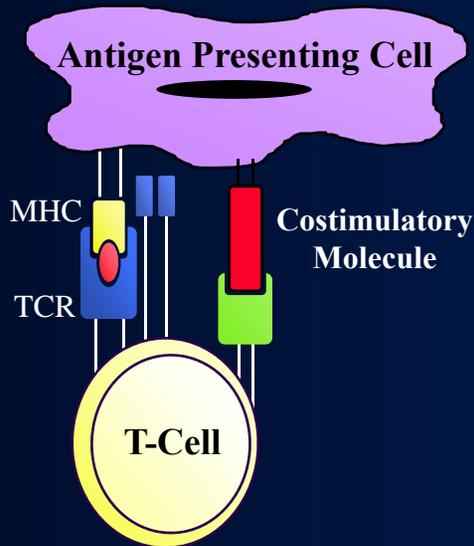
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5. **Cytokines — biologic adjuvants**

T-cell Costimulation

- **Costimulatory molecules are expressed on professional antigen presenting cells (APC): dendritic cells, B cells, macrophages, monocytes**
- **Costimulatory molecules are not expressed on the vast majority of solid tumors**

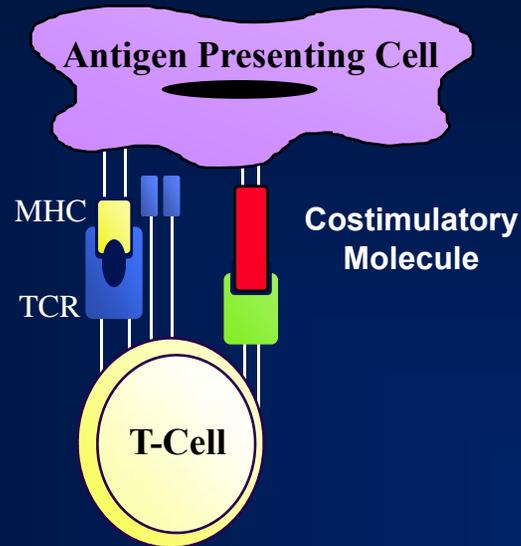
T-Cell Dependence on Costimulation

Signal 1 + Signal 2



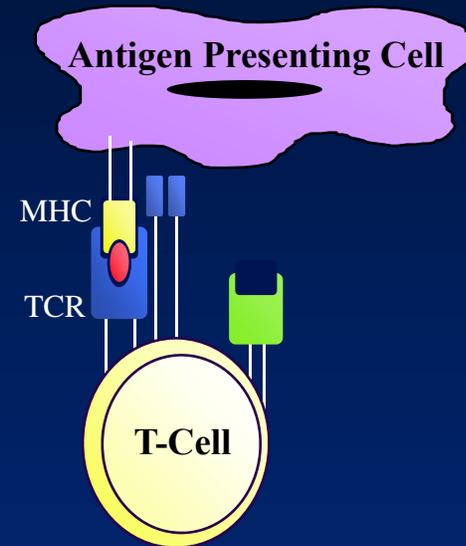
Activation of Antigen-Specific T-cells

No Signal 1



**Clonal Anergy
Apoptosis
Ignorance**

No Signal 2

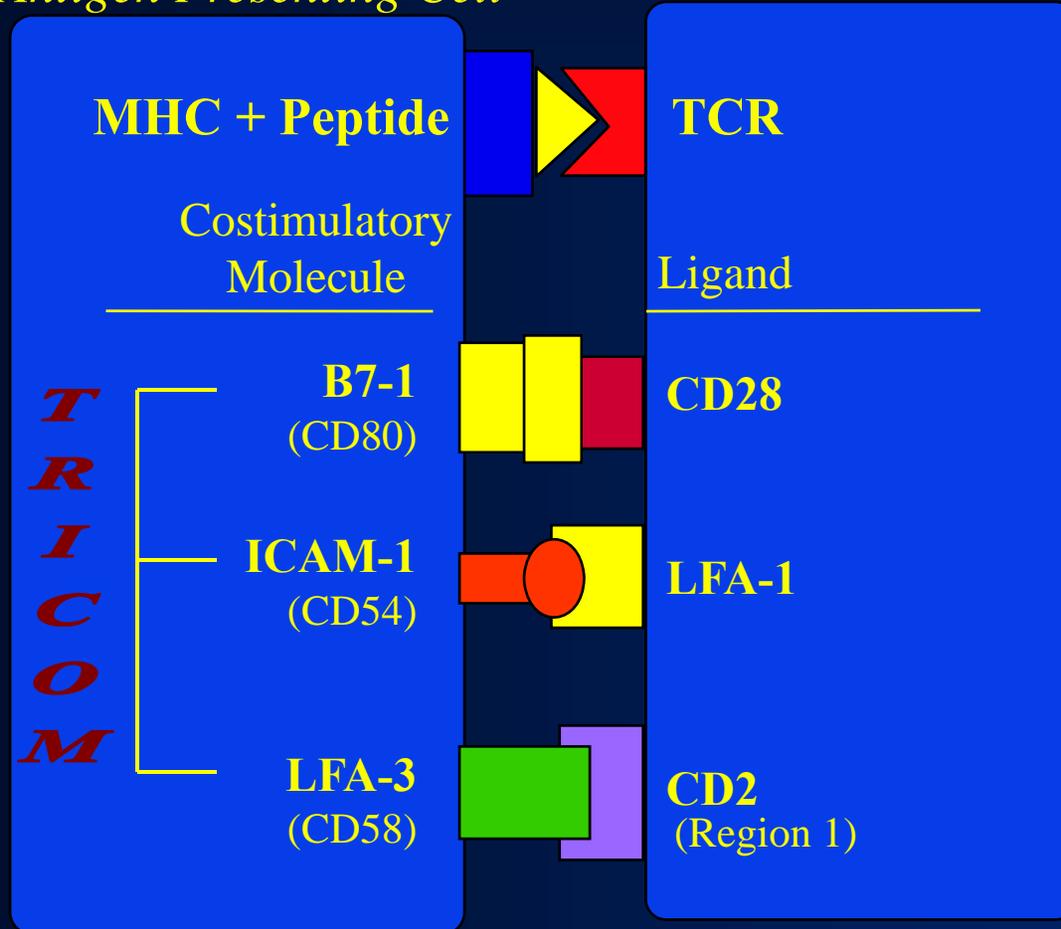


**Clonal Anergy
Apoptosis
Ignorance**

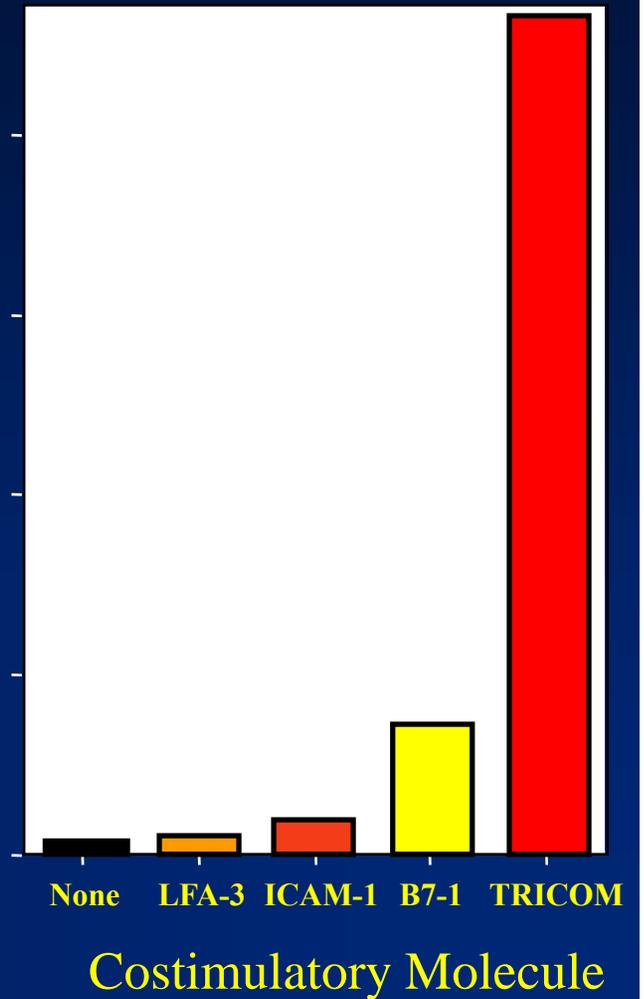
T-cell Activation and Costimulatory Molecules

Antigen Presenting Cell

T-Cell



T-cell Activation (CPM x 10⁵)



TRICOM

TRIad of COstimulatory Molecules

Costimulatory Molecule

B7-1 (CD80)

ICAM-1 (CD54)

LFA-3 (CD58)

Ligand on T cell

CD28/CTLA-4

LFA-1

CD2

e.g. , rV-Tricom = rV-B7-1/ICAM-1/LFA-3

avi-Tricom = avi -B7-1/ICAM-1/LFA-3

rV-CEA(6D)/Tricom = rV-CEA(6D)/B7-1/ICAM-1/LFA-3

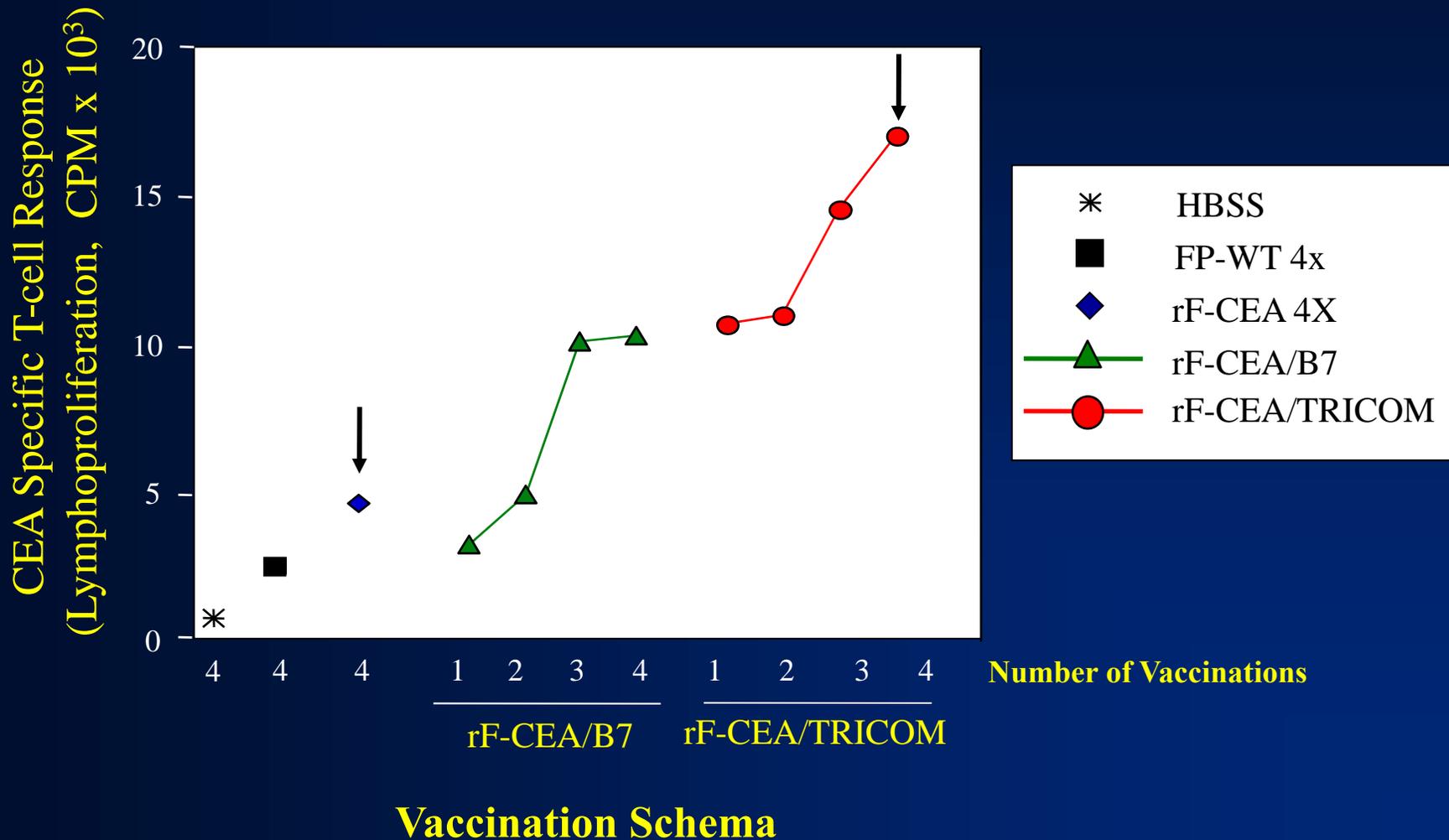
avi-CEA(6D)/Tricom = avi-CEA(6D)/B7-1/ICAM-1/LFA-3

rV-PSA(3A)/Tricom = rV-PSA(3A)/B7-1/ICAM-1/LFA-3

avi-PSA(3A)/Tricom = avi-PSA(3A)/B7-1/ICAM-1/LFA-3

avi = rF = recombinant avipox (fowlpox)

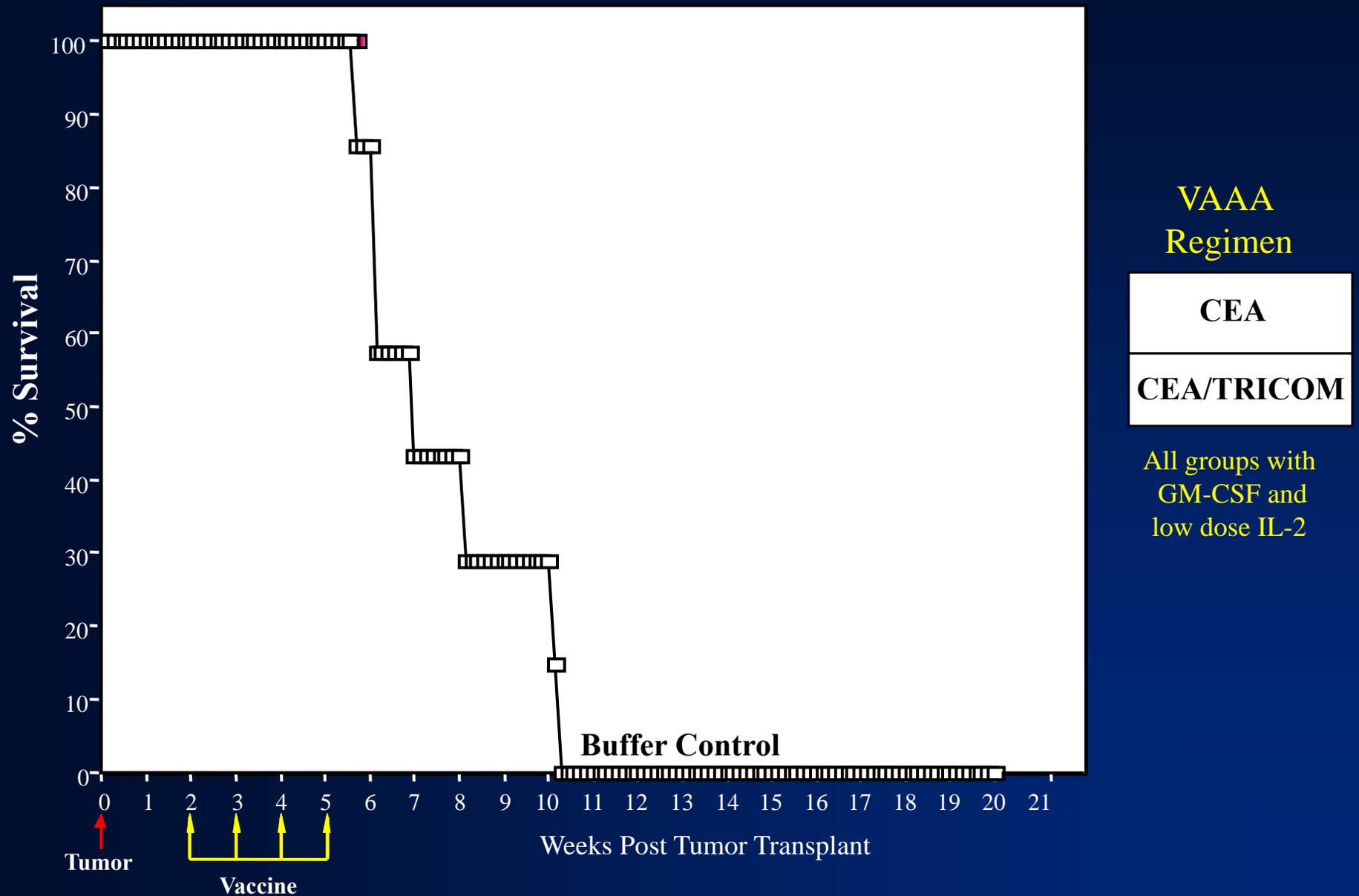
CEA-Specific T-cell Responses Following Vaccination with rF-CEA, rF-CEA/B7-1, or rF-CEA/TRICOM



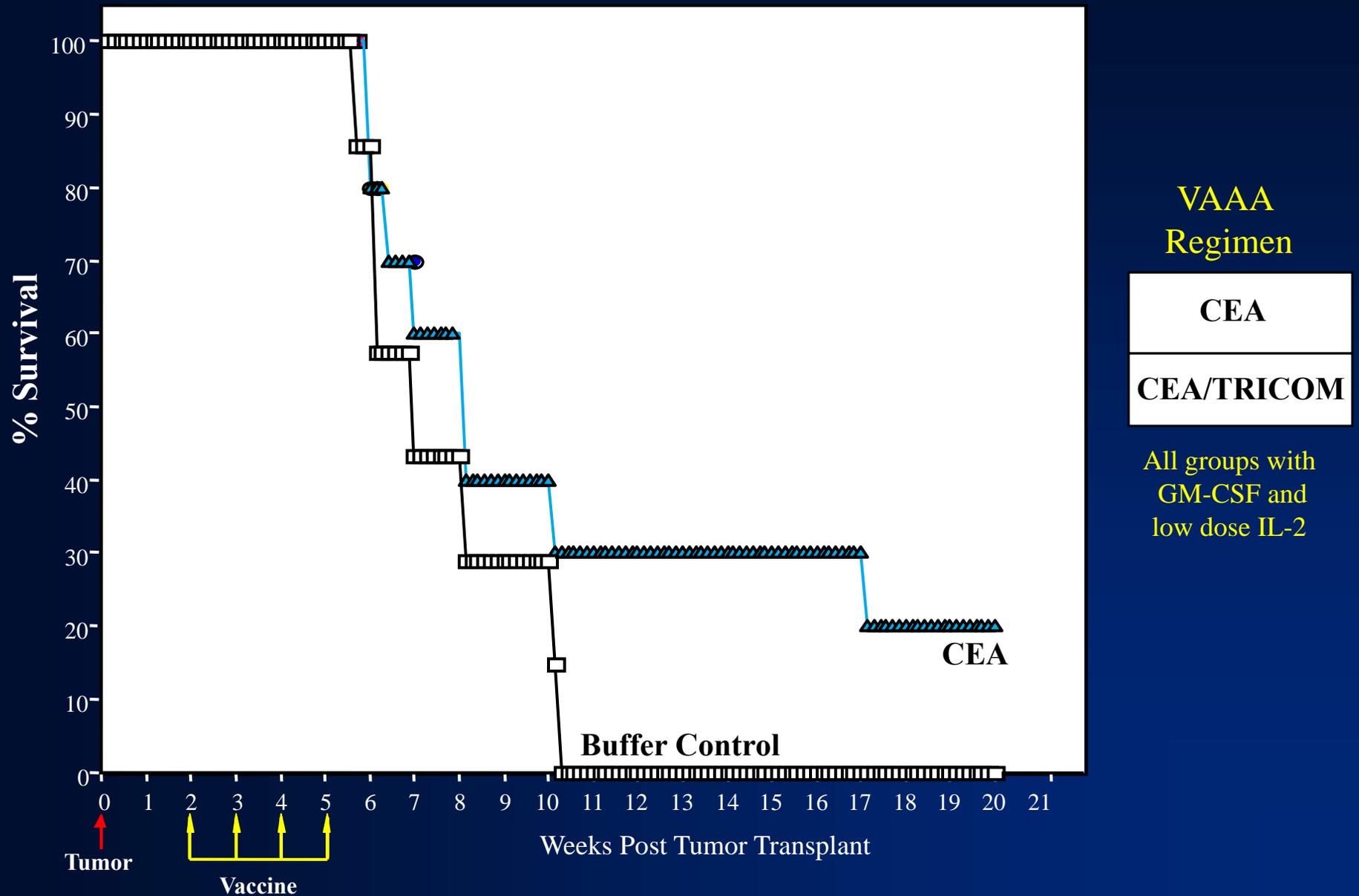
Vaccine Tumor Therapy Model

- **CEA Transgenic (Tg) mice**
 - CEA expressed in fetal gut and adult GI tissues (similar to humans)
- **Experimental Metastases**
 - MC-38 colon carcinoma cells expressing CEA
- **Begin vaccine therapy at **day 14** post-tumor transplant**

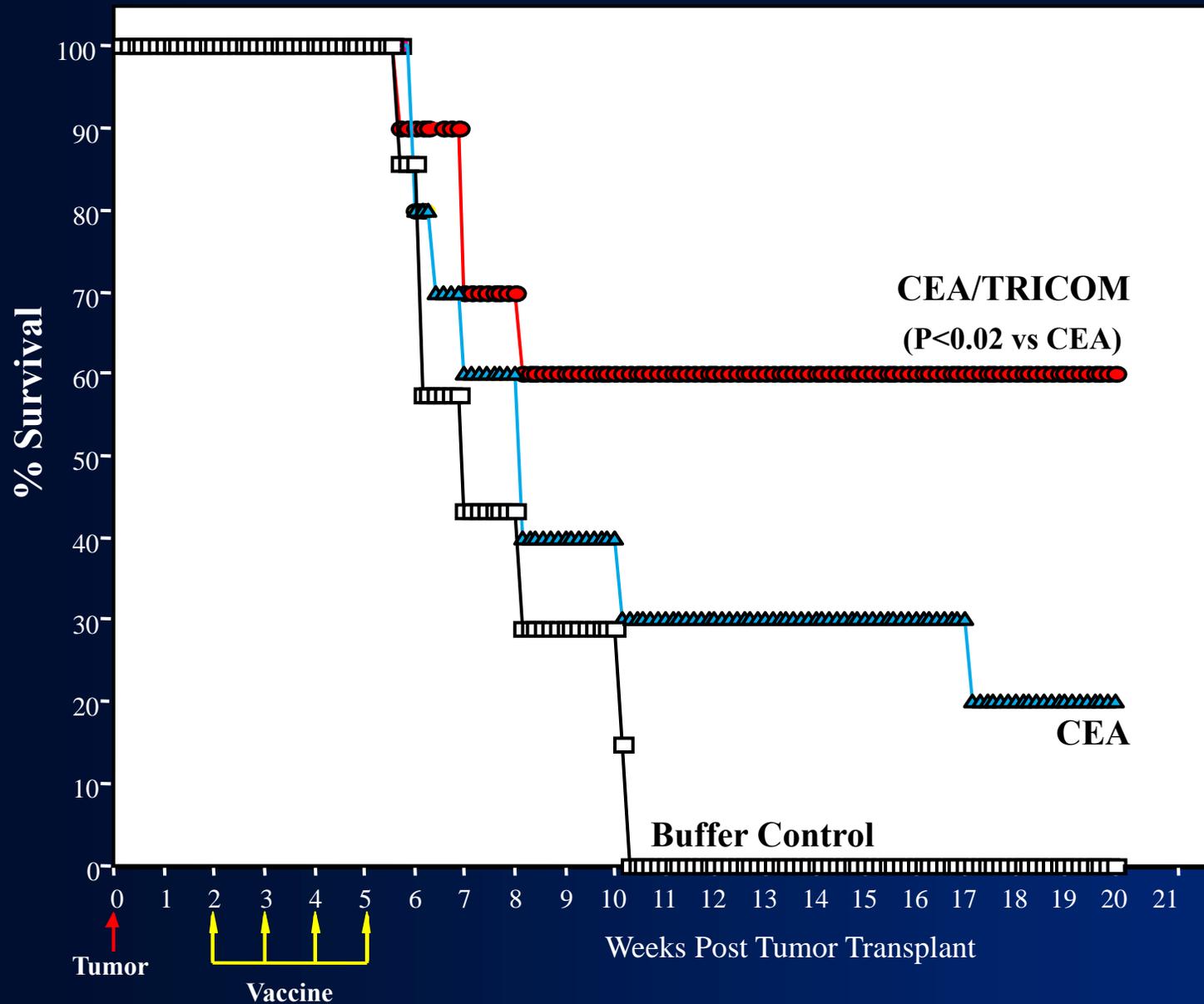
Therapy of 14-Day Established CEA⁺ Experimental Metastases in CEA-Tg Mice Using CEA/TRICOM Vectors



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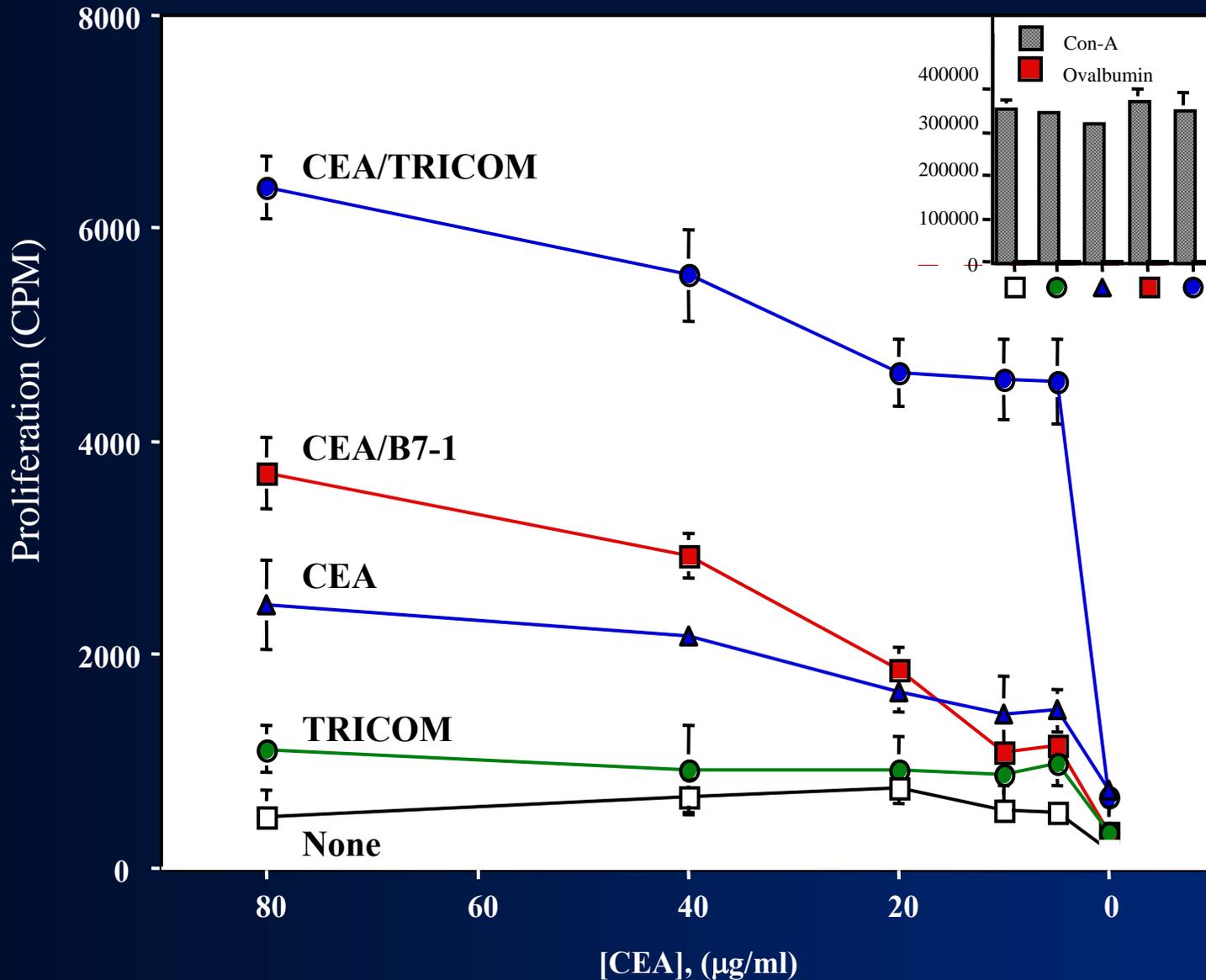


VAAA
Regimen

CEA
CEA/TRICOM

All groups with
GM-CSF and
low dose IL-2

CEA-specific Lymphoproliferation of T Cells from CEA-Tg Mice Vaccinated with TRICOM Vectors



VAAA Regimen

CEA
CEA/B7-1
CEA/TRICOM
TRICOM

All groups with GM-CSF and low dose IL-2

Clinical Trials

Phase I Georgetown, J. Marshall

- Avipox-CEA(6D)-TRICOM (A) -4 vaccinations
- rV-CEA(6D)-TRICOM (V) -1 vaccination
 - boost with Avipox-CEA(6D)-TRICOM -3 vaccinations

Phase I Fox Chase, M. von Mehren

- Avipox-CEA(6D)-TRICOM
 - + GM-CSF vs. avipox GM-CSF

Phase I Duke, K. Lyerly

- Avipox-CEA(6D)-TRICOM
 - infection of dendritic cells

CEA-TRICOM Phase I Trial: Patient Demographics

<u>Characteristics</u>	<u>Number of Patients</u>
Total	58
<i>Prior therapy</i>	
<u>Chemotherapy</u>	
0 regimen	3
1 prior regimens	7
2 prior regimens	12
> 2 prior regimens	36
Radiation	16
<i>Primary site</i>	
Colorectal	34
Lung	7
Breast	4
Thyroid	1
Unknown primary	1
Renal	1
Other GI	10
HLA-A2 +/-	31/27

CEA-TRICOM Phase I Trial (Georgetown U., J. Marshall, P.I.)

- **Stage 1: F= Fowlpox-CEA (6D)-TRICOM** (F)
 - Cohort 1: F-F-F-F 4 x 10⁶ pfu 3 pts
 - Cohort 2: F-F-F-F 4 x 10⁷ pfu 3 pts
 - Cohort 3: F-F-F-F 4 x 10⁸ pfu 10 pts (6 A2+)
- **Stage 2: V= vaccinia-CEA (6D)-TRICOM** (V)
 - Cohort 4: V-F-F-F 4 x 10⁶ pfu 3 pts
 - Cohort 5: V-F-F-F 4 x 10⁷ pfu 3 pts
 - Cohort 6: V-F-F-F 4 x 10⁸ pfu 10 pts (6 A2+)
- **Stage 3: GM= GM-CSF 100 mcg subq D1-4**
 - Cohort 7: V-F-F-F + GM 10 pts (6 A2+)
- **Stage 4: Split dose of rF + GM-CSF 100 mcg sq bilaterally**
 - Cohort 8: V-F-F-F +GM 10 pts (6 A2+)

CEA-TRICOM Phase I Trial

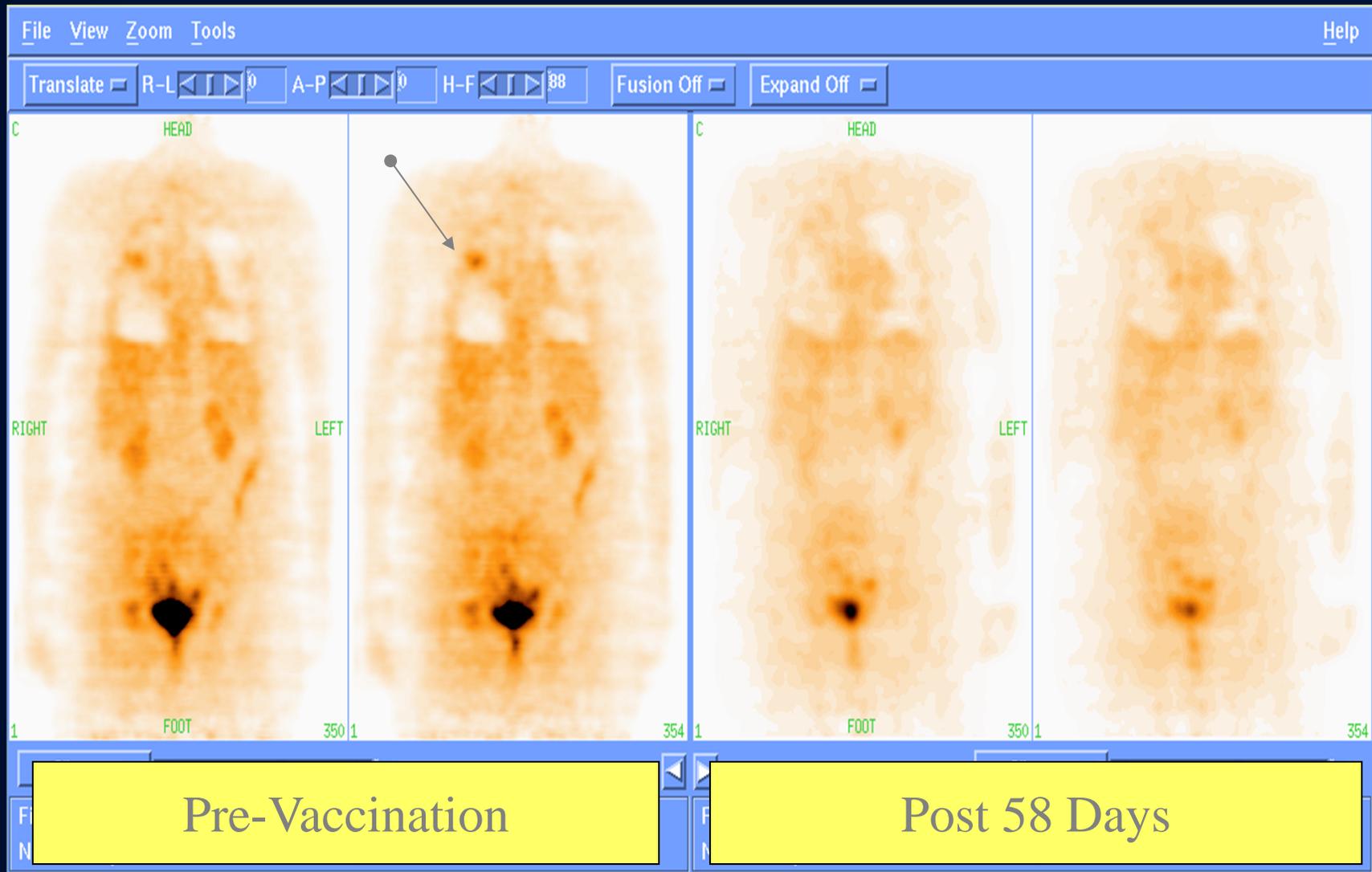
Toxicity: no dose limiting toxicity

no Grade II, III, or IV toxicities attributed to vaccine

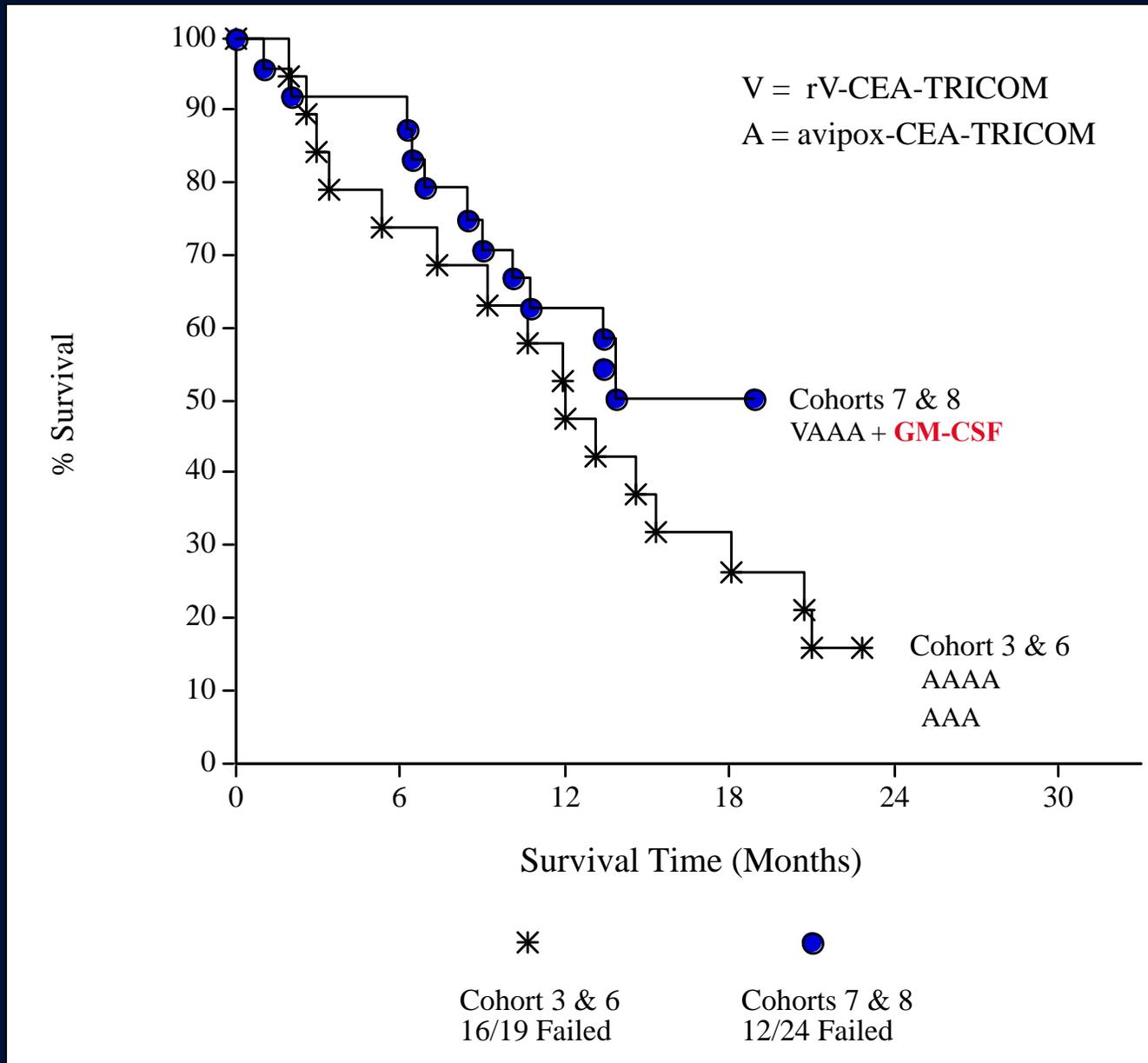
Clinical Results

- 23 patients (40%) had stable disease > 4 months
- 11 patients had stable or decreasing serum CEA / 19.9
- 1 pathologic complete response—lung cancer patient
- Patients who remained stable after 6 monthly vaccinations went on to vaccinations every 3 months
 - 6/12 patients who progressed after being given vaccines every 3 months restabilized after monthly vaccinations

PET Images of Patient after 2 Vaccinations with Avipox-CEA(6D)/TRICOM

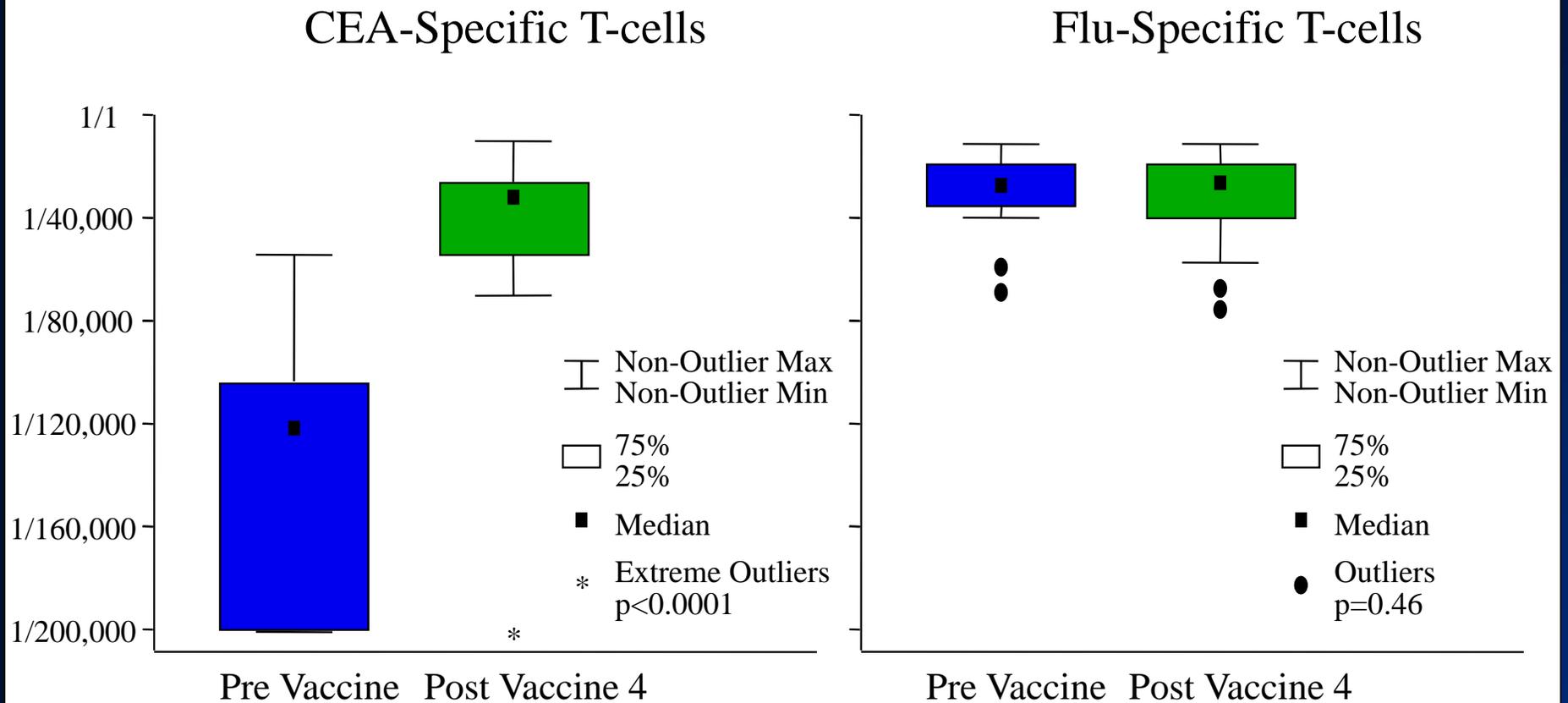


CEA-TRICOM Phase I Trial: Survival by Cohort



CEA-TRICOM Phase I Study

CEA-Specific and Flu-Specific T-Cell Responses



CEA-TRICOM Phase I Trial

CEA-Specific T-Cell Responses in PBMC* (HLA-A2 patients):

Increases in 13/16 patients

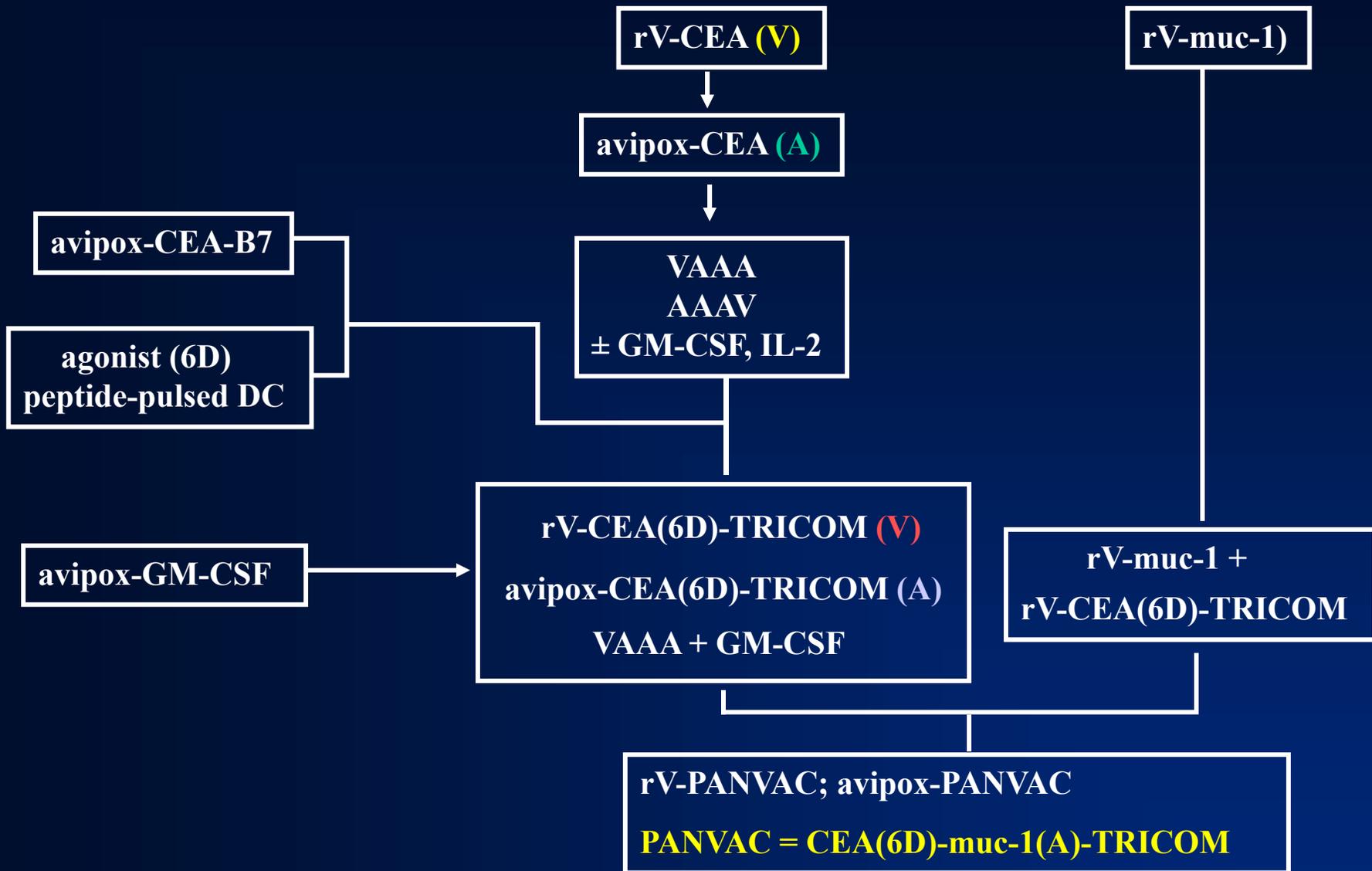
Using 1/30,000 as a cut-off after 4 vaccinations:

- correlation with progression-free survival ($p = 0.04$)
- correlation with overall survival ($p = 0.06$)

- 83% of patients who had a CEA-specific T-cell response of $< 1/30,000$ after 4 vaccinations were alive at 1 year, vs. 41% of patients whose CEA precursors were $>1/30,000$ after 4 vaccinations

* Using ELISPOT assay for IFN- γ production by PBMC in response to CEA-specific peptide post 4 vaccinations vs. pre-vaccination.

Clinical Development of Vector-Based Vaccines

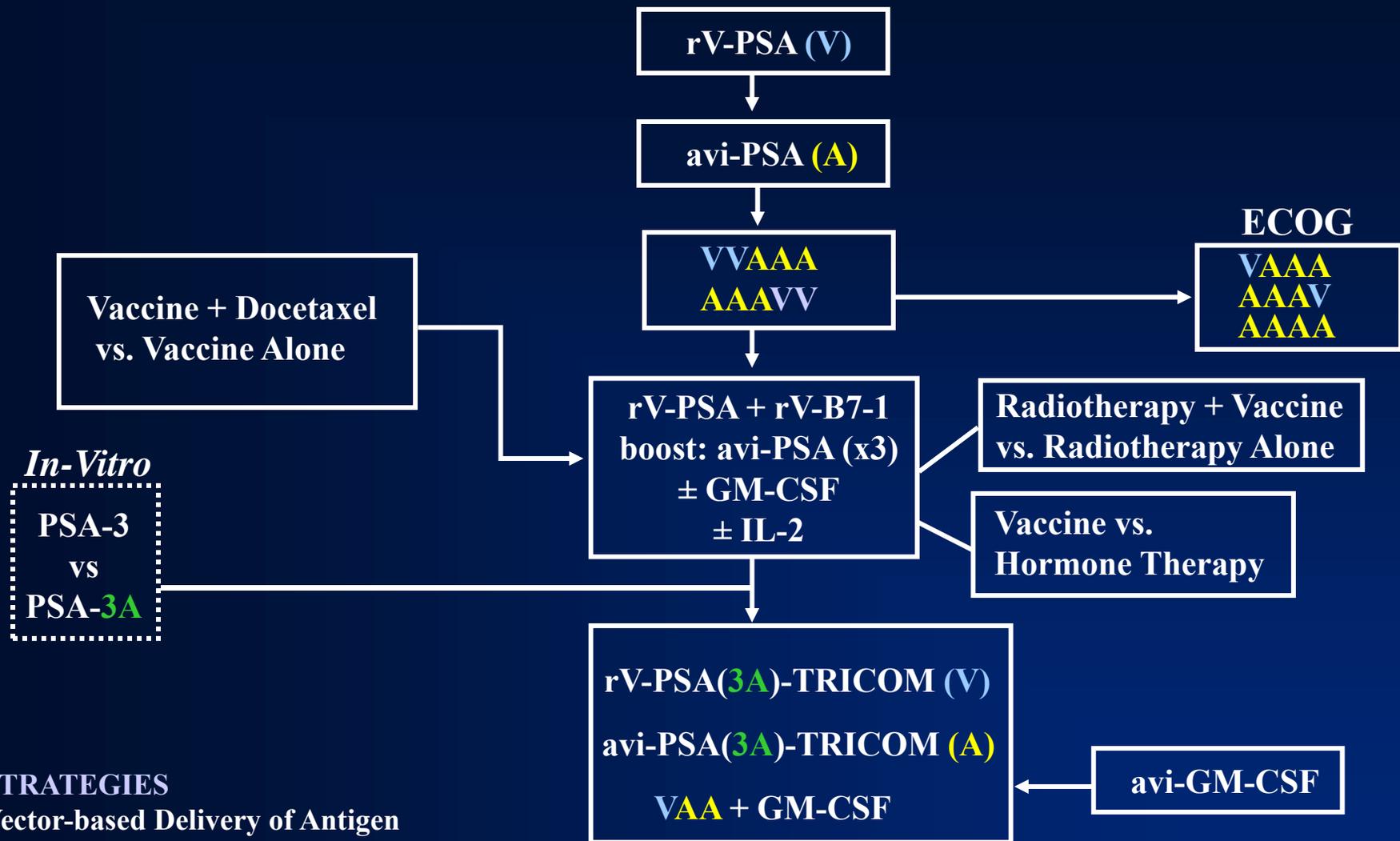


TRICOM = B7.1/ICAM-1/LFA-3

CEA(6D) = CEA gene containing agonist epitope

muc-1(A) = muc-1 gene containing agonist epitope

PSA Vaccine Clinical Development Plan



STRATEGIES

Vector-based Delivery of Antigen
(**rV, avi**)

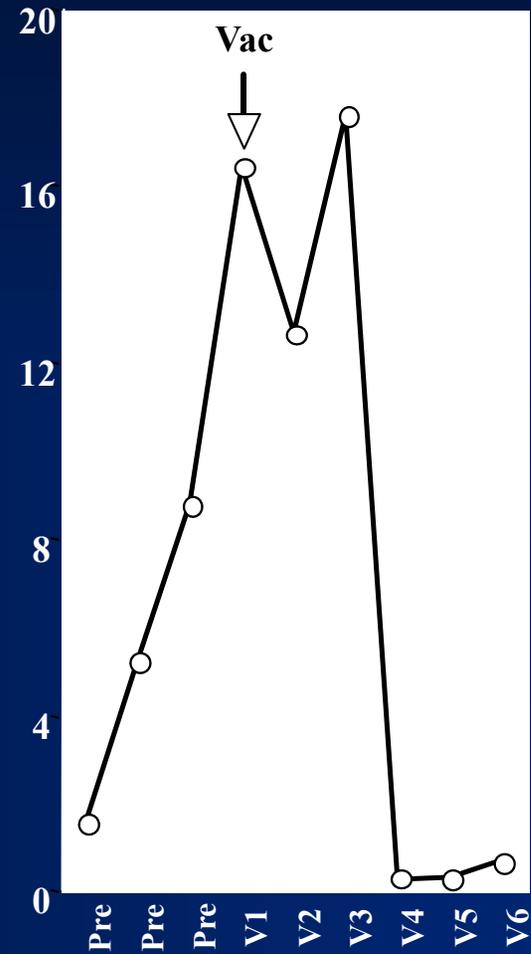
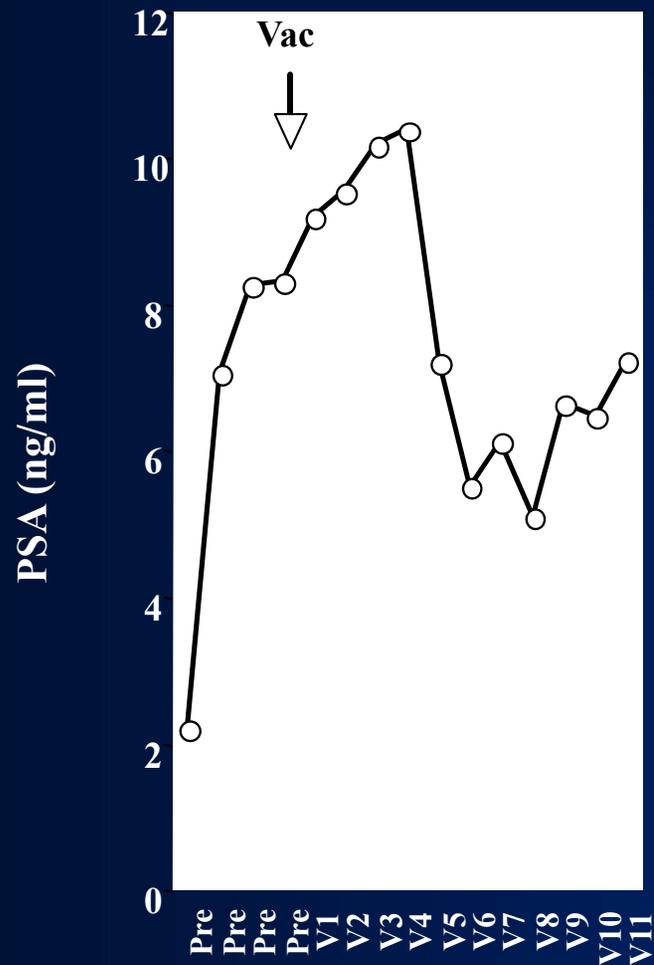
Diversified Prime and Boost (**VAAA**)

Costimulation (**B7-1 -> TRICOM**)

Agonist (**3A**)

Cytokine (**GM-CSF**)

Serum PSA Levels in Hormone Refractory Patients Vaccinated with rV-PSA + rV-B7-1 and Boosted with Avipox-PSA



Antigen Cascade – Gulley Trial: rV-PSA + rV-B7.1 → rF-PSA

<u>Patient</u>	<u>Sample</u>	<u>Flu</u>	<u>PSA3</u>	<u>PSMA</u>	<u>PAP</u>	<u>PSCA</u>	<u>MUC-1</u>
Pt 1	pre vac	1/50,000	1/200,000	<1/200,000	<1/200,000	1/200,000	1/200,000
	post 3	1/37,500	1/42,857	<1/200,000	<1/200,000	<1/200,000	<1/200,000
Pt 2	pre vac	1/21,429	<1/200,000	<1/200,000	<1/200,000	<1/200,000	<1/200,000
	post 3	1/21,429	1/60,000	1/200,000	1/85,714	<1/200,000	<1/200,000
Pt 3	pre vac	1/85,714	<1/200,000	<1/200,000	<1/200,000	<1/200,000	<1/200,000
	post 3	1/54,545	1/75,000	<1/200,000	<1/200,000	<1/200,000	1/40,000
Pt 4	pre vac	1/20,690	<1/200,000	<1/200,000	<1/200,000	<1/200,000	<1/200,000
	post 3	1/22,222	1/66,667	1/85,714	<1/200,000	<1/200,000	1/60,000
Pt 5	pre vac	1/75,000	<1/200,000	<1/200,000	<1/200,000	<1/200,000	<1/200,000
	post 3	1/100,000	1/85,714	<1/200,000	1/85,714	1/85,714	1/23,077

Future Studies

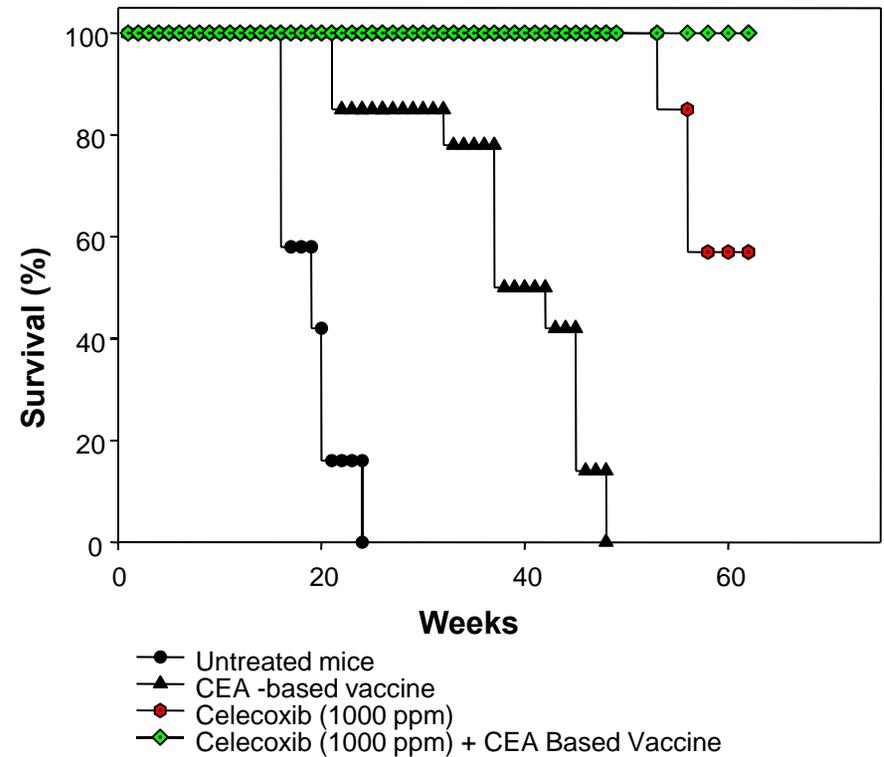
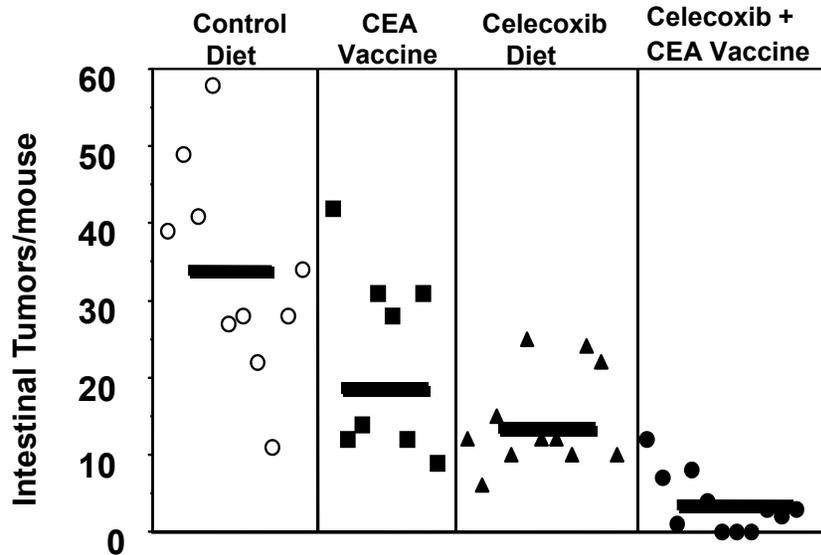
- **The use of TRICOM vaccines in combination with conventional therapies**
 - radiation
 - selected drugs

Tumor Prevention/Therapy Model

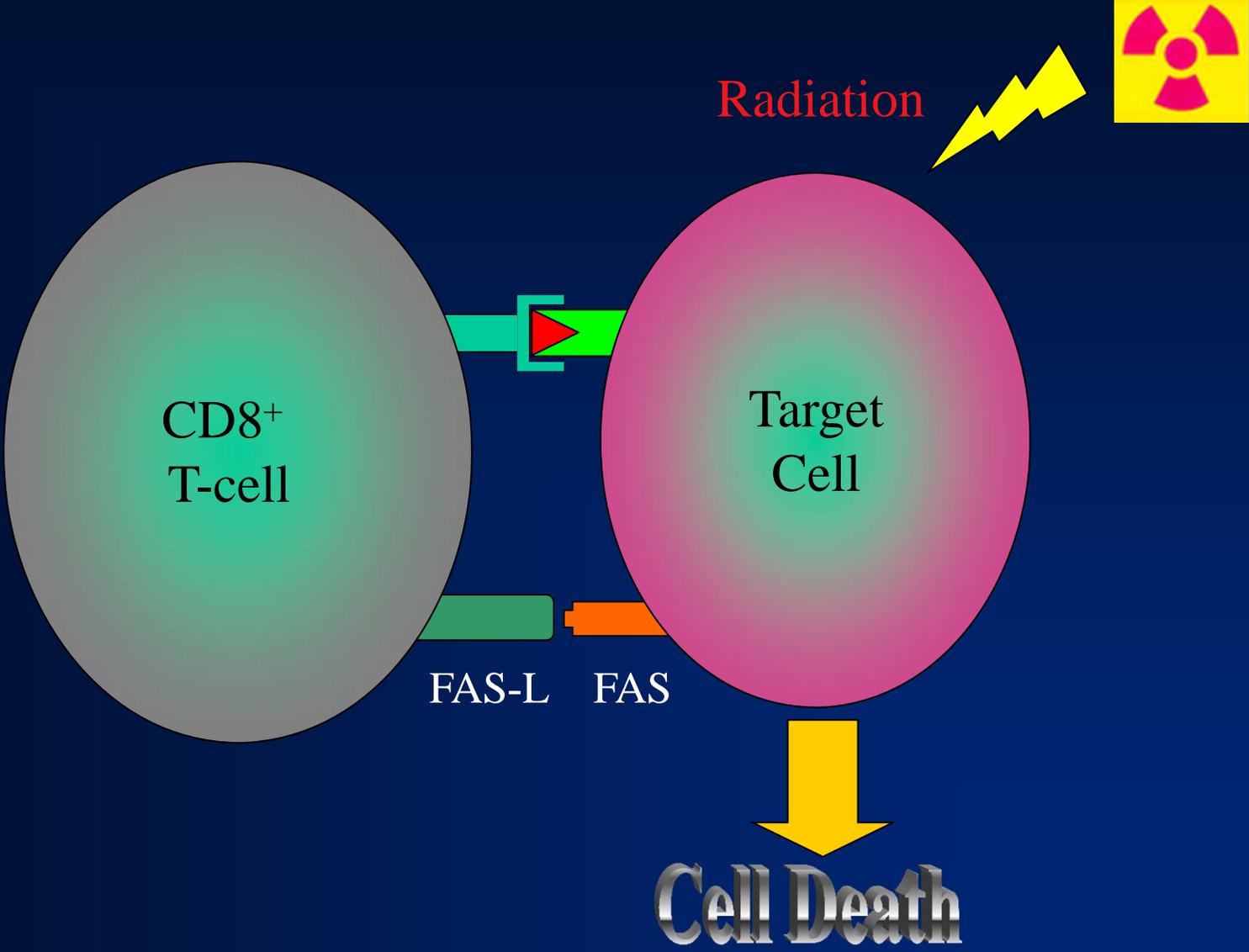
- **CEA-Transgenic Mice: CEA is a self antigen**
- **APC/min⁺ mice: Develop numerous **spontaneous** colon tumors (polyps) and die from anemia**
- **CEA-Tg x APC/min⁺**
 - **develop CEA⁺ spontaneous tumors**

COX-2 inhibitors (Celecoxib) FDA approved for the treatment of polyps

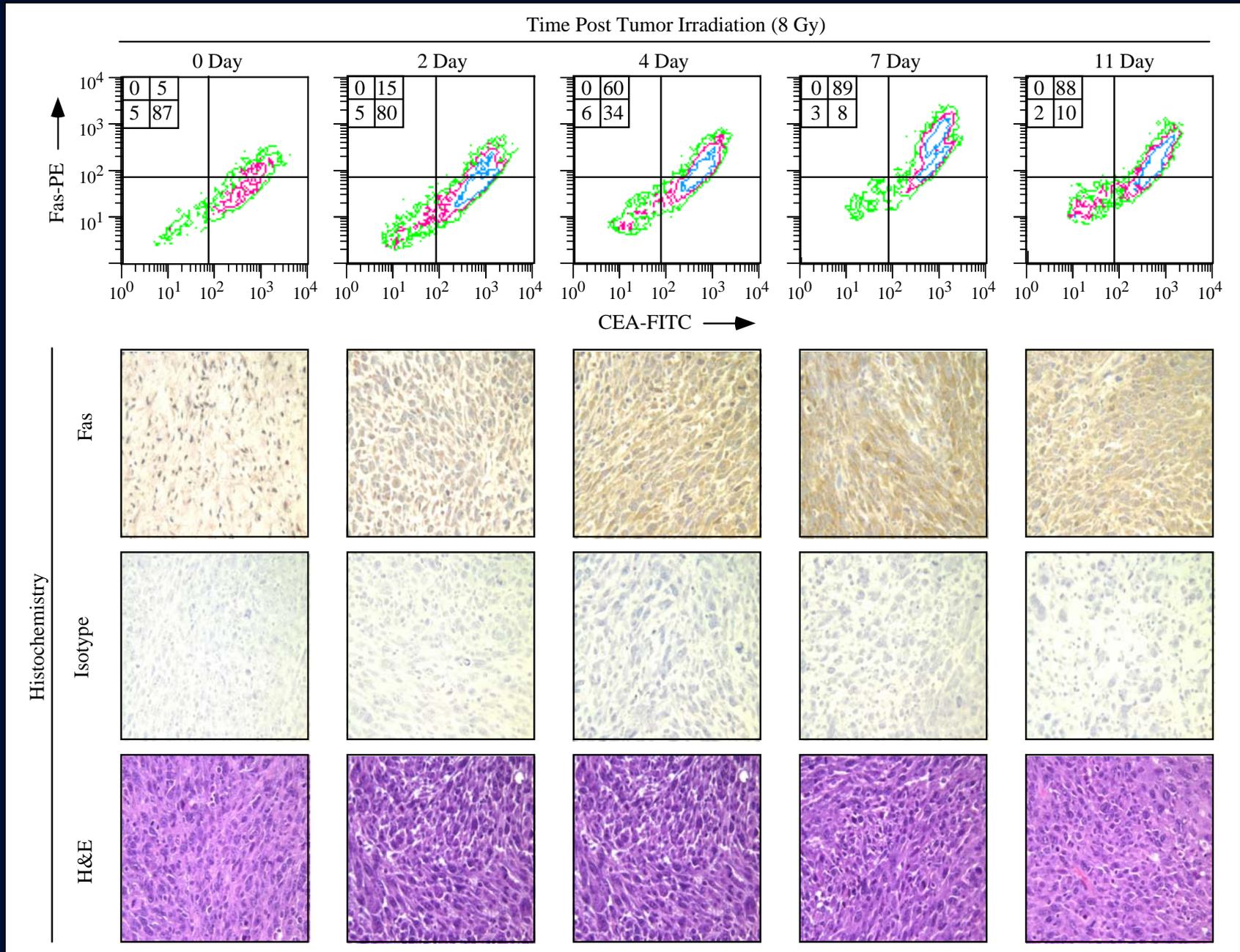
Anti-tumor Effects of Spontaneous Tumors in CEA.Tg/MIN Mice Using a CEA-TRICOM Vaccine and Celecoxib



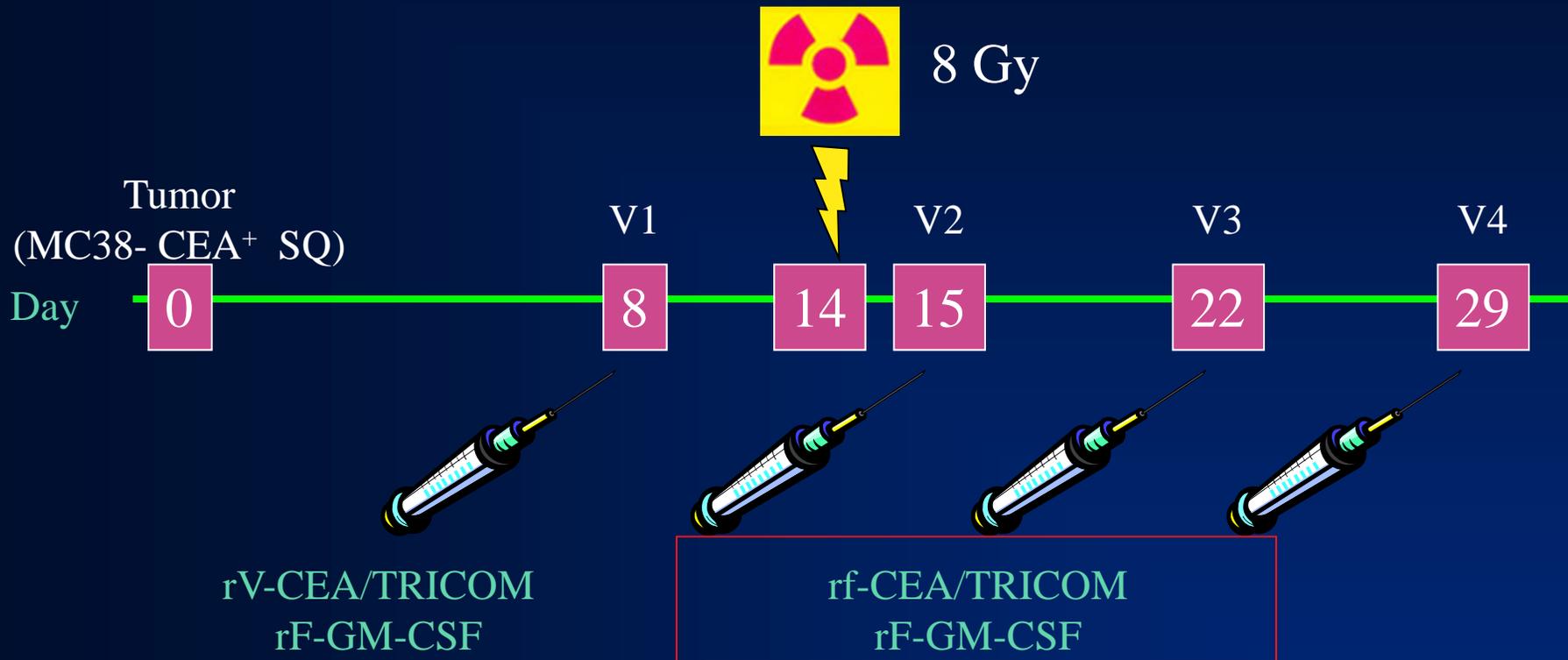
Radiation-Enhanced Antigen-Specific Lysis of Tumor Cells



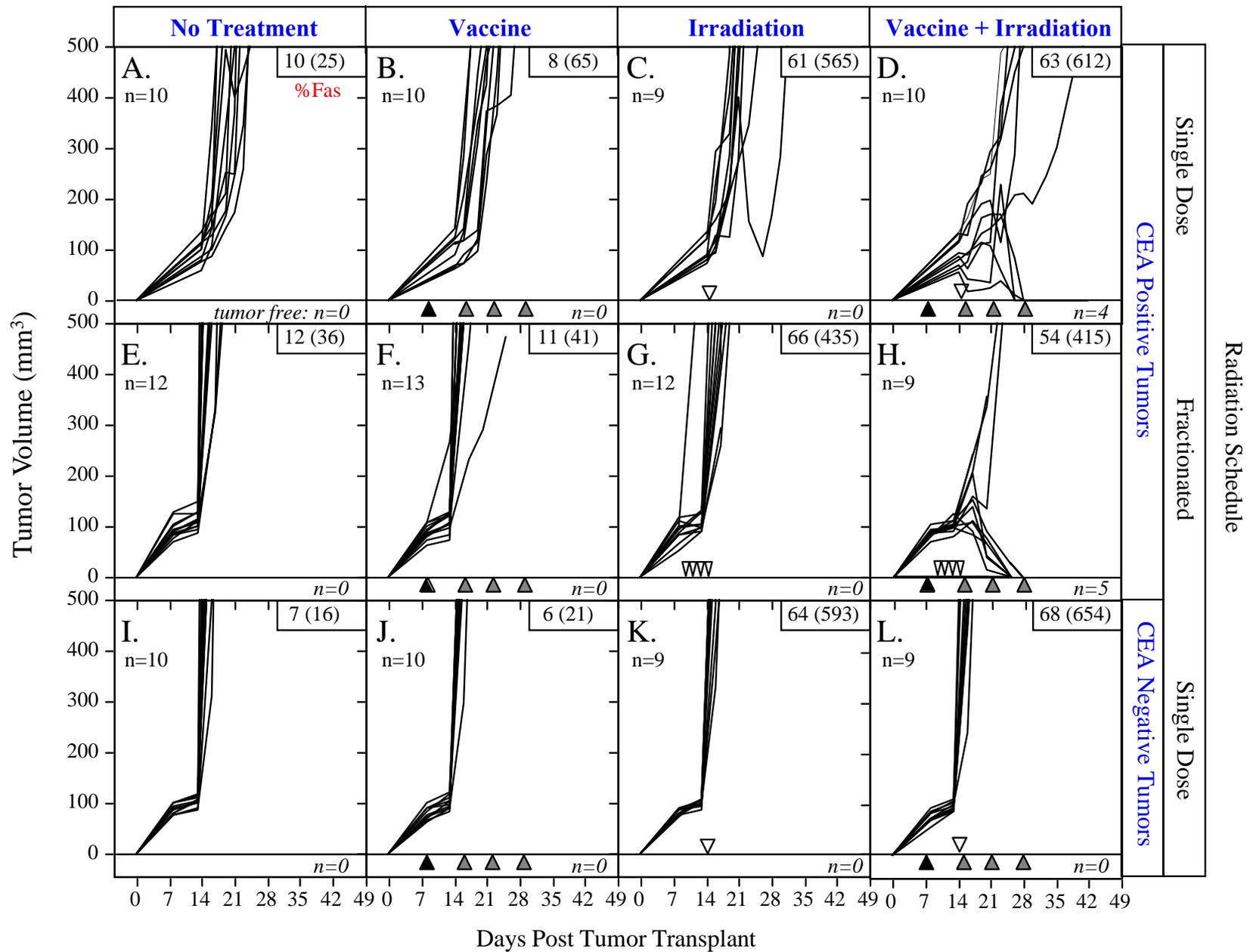
Persistence of Fas Upregulation on MC38-CEA⁺ Tumors After External-Beam Irradiation



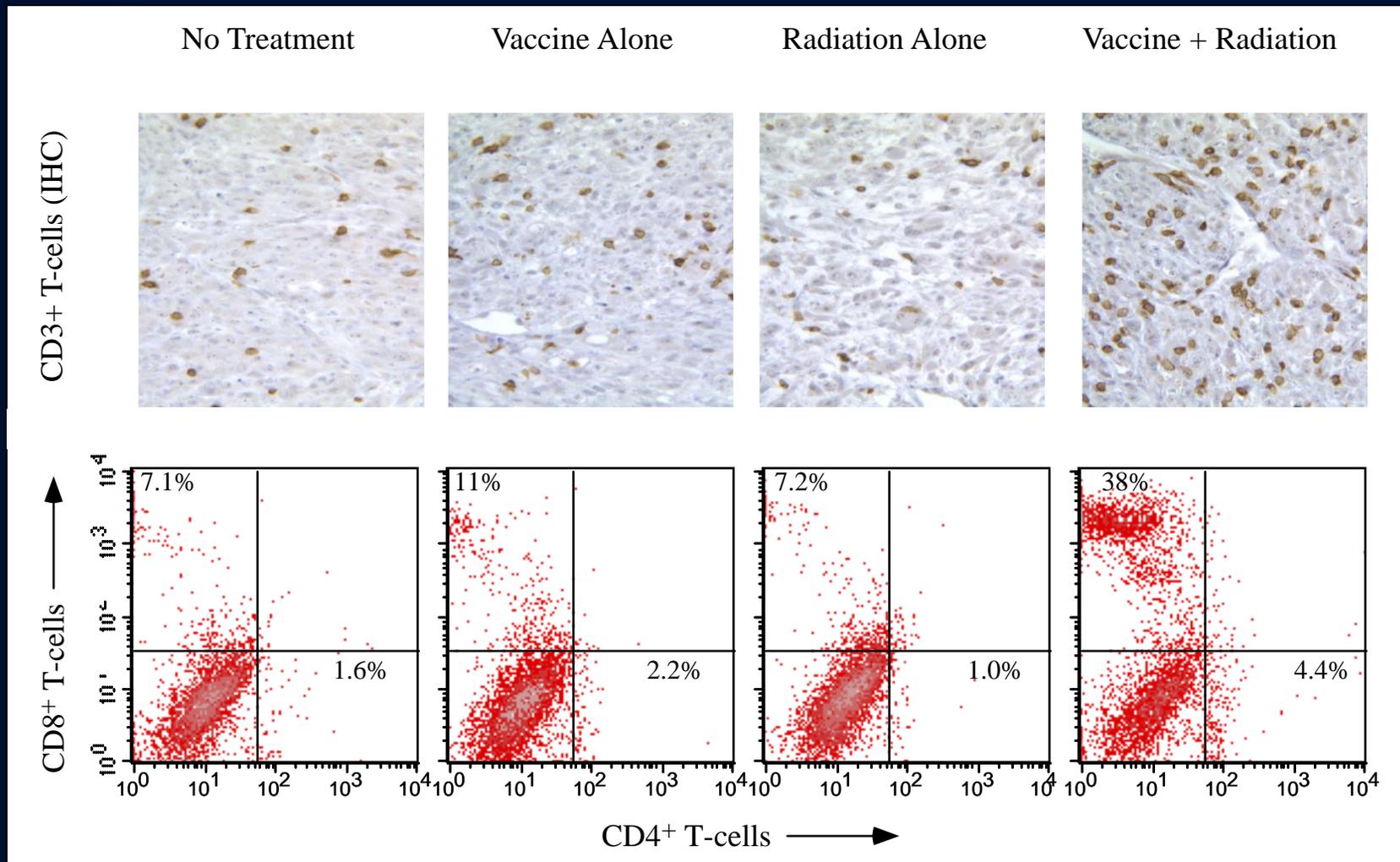
Schema of Combination Vaccine Therapy and Radiation



Combination Therapy : Vaccine + External Beam Radiation



Vaccine + Radiation: Tumor Infiltrating Cells



Strategic Plan for Clinical Trial Development Combinatorial Vaccine Therapy

CEA-TRICOM Vaccines (V, A)

Phase I — Pancarcinoma

CEA-muc-1-TRICOM Vaccines

(V, A): PANVAC

Phase I — Pancarcinoma

Phase II Trials:

Lung Cancer

Stage III

Radiation/

Chemo

± Vaccine

Breast Cancer

Metastatic

Docetaxel

± Vaccine

Colon Cancer

Stage 4: Liver Met.

Vaccine

± Radiation

± COX-2 Inhibitor

Pancreatic Cancer

Therion IND

Phase I/II/III

In Conclusion

Hypothesis-Driven Preclinical Studies Have Been Translated to Science-Based Clinical Trials:

- **Viral Vector-Based Vaccines**
- **Diversified Prime and Boost Vaccination Schema**
- **T-Cell Costimulation (TRICOM)**
- **Agonist Epitope Enhancement**
- **Cytokines as Biologic Adjuvants**
- **Multi-Tumor Antigen Vaccine Constructs**

Strategic Plan

- **Integration of Recombinant Vaccine Regimens with Local Tumor Irradiation and Chemotherapy**
- **Continue Programmatic Efforts: Intramural Research Program, 8 Cancer Centers, Cooperative Groups, Private Sector**